Table 1: Function and protein content of left ventricle tissue from rat hearts subjected to ischemia and ischemia/reperfusion

| Experimental Protocol[a] | | | Skinned Fiber Experiment[b] | | Quantity of Protein Present in Tissue[c] | | | | | | Quantity of Protein in Effluent[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ischemia (minutes) | reperfusion (minutes) | $pCa_{50}$ | maximum force (mg/mg) | Troponin I | α-actinin | TM | MLC1 | OSC protein[f] | MLC2 | relative peak area at 23 min |
| 1) | 0 | 45[e] | 5.82±0.01 | 5190±72 | 0.28±0.05 | 0.16±0.01 | 0.29±0.05 | 0.13±0.03 | ND | 0.11±0.02 | - |
| 2) | 15 | 0 | 5.92±0.01 | 5830±21 | 0.28±0.02 | 0.17±0.02 | 0.24±0.01 | 0.13±0.01 | ND | 0.05±0.01 | NA |
| 3) | 15 | 45 | 5.93±0.02 | 2790±36 | 0.37±0.02 | 0.10±0.04 | 0.24±0.01 | 0.18±0.01 | 0.05±0.01 | 0.09±0.01 | + |
| 4) | 60 | 0 | 5.86±0.02 | 2860±52 | 0.33±0.03 | 0.12±0.01 | 0.24±0.03 | 0.23±0.05 | 0.05±0.01 | 0.10±0.03 | NA |
| 5) | 60 | 45 | 6.03±0.01 | 1670±12 | 0.51±0.07 | 0.10±0.02 | 0.29±0.02 | 0.30±0.4 | 0.09±0.02 | 0.19±0.05 | +++ |

[a] All rat hearts underwent 15 minutes of equilibration prior to starting the experimental protocol. When required, isoproterenol was added to the perfusated during the final 5 minutes of the equilibrium period.

[b] The $pCa_{50}$ (-log concentration of calcium required to induce half of the $Ca^{2+}$-dependent change in force) and maximum force (mg/mg) was determined from curve fitting the data in Figure 1. Force produced by a skinned fiber (mg) per total protein content of the corresponding skinned fiber (mg) with respect to changing calcium concentrations is plotted. This quantifies the amount of force exhibited by each fiber taking into account the size of the fiber.

[c] Quantity of TnI and α-actinin determined from densiometry measurements (± STD) from 12.5% SDS-PAGE. ND = not detected

[d] The quantity of protein present in the effluent was assigned a grading system (+++ most, ++ intermediate and + least) based on HPLC analysis of effluent samples. NA stands for not applicable, protocols were reperfusion was not done.

[e] Control conditions which are 0 minutes ischemia followed by 45 minutes perfusion.

[f] OSC = ATP synthase oligomycin sensitivity conferring protein.

Table 2: Identification of proteins affected by ischemia and ischemia/reperfusion by amino acid sequencing

| Amino Acid Sequence | Protein | Residue Number | First Identified Amino Acid (pmole) | Tissue Sampled |
|---|---|---|---|---|
| XXKKPE(P/A)KADDA | myosin light chain1 | 1-12 | 2.6 | global ischemia myofibrils & 60I/45RP tissue |
| XPAPAAAPAAAP | myosin light chain1 | 20-31 | 6.0 | global ischemia myofibrils |
| XKVALGAXGGI | malate dehydrogenase | 1-11 | 3.2 | 60I/45 RP tissue |
| XXLKDITRRLKSI | ATP g synthase chain | 1-13 | 4.5 | 60I/45RP tissue |
| XXKLVRPPVQ | ATP synthase oligomycin conferring protein | 1-10 | 2.3 | 60I/45RP tissue |
| XAHKSEIAHR | serum albumin | 1-10 | 12.4 | 60I/45RP effluent |
| XPS(R/L)KFFVGGN | triose phosphate isomerase | 1-11 | 9.9 | 60I/45RP effluent |

**Table 3: Progressive Alteration of TnI with Increasing Severity of Ischemia.**

| Ischemia/Reperfusion Induced TnI Product | | A. Left Ventricle Tissue: Percentage of Total TnI* 0/45‡ | 15/45 | 60/45 | B. Anti-TnI Affinity Chromatography: Percentage of Total TnI† Peak 2 | Peak 3 |
|---|---|---|---|---|---|---|
| Covalent Complexes§ | | 0 | 16.9% | 3.1% | N/D// | N/D |
| rcTnI | | 94.4% | 52.2% | 35.3% | 71.7% | 91.4% |
| rcTnI Degradation Products | ~22 kDa | 5.6% | 24.1% | 21.3% | 25.5% | 6.4% |
| | ~16 kDa | 0 | 0 | 15.1% | 2.8% | 2.2% |
| | ~15 kDa | 0 | 0 | 17.2% | N/D | N/D |

* The ischemia/reperfusion-induced modified TnI products observed in urea T-PAGE separated left ventricular tissue which underwent either 0/45, 15/45, or 60/45 (Figure 9) were quantified from 8I-7 MAb Western blots (Figure 9A). The quantity of each TnI component was determined as a percentage of the total TnI (intact and modified) present in each tissue sample. Only those products which are positively identified in Table 4 are included here, identified by their apparent molecular weight (Figure 9A).

† The ischemia/reperfusion-induced modified TnI products observed from 8I-7 MAb affinity chromatography of 60/45 left ventricular tissue (Figure 11) were quantified from 8I-7 MAb Western blots (Figure 11B), and the amount of each TnI component determined as a percentage of the total in each sample.

‡ Control tissue, which experienced no ischemic episode, but 45 minutes of reperfusion.

§ The quantity of the two TnI-containing covalent complexes combined.

// Quantities less than 2% of total TnI could not be accurately determined.

**Table 4: Identification of Ischemia-Induced TnI Products by Mass Spectrometry.**

| Ischemia/Reperfusion-Induced TnI Product * | | Source ¶ | Immunoreactivity with MAb's † | | | | Alk. Urea PAGE ‡ | Putative Identification § | Observed Mass (Da ± S.E.) | Theoretical Mass (Da) // |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 8I-7 | 3I-35 | AM-IN | TnT | | | | |
| Covalent Complexes | ~66 kDa | peak 3 | + | ± | + | + | - | rcTnI(1-193) /TnT(191-298) | 32 872 ± 9 # | 32 871 |
| | ~55 kDa | peak 2 | + | ± | + | - | + | rcTnI(1-193) /TnC(1-94) | 32 734 ± 14 # | 32 730 |
| rcTnI Degradation Products | ~22 kDa | peak 2 | + | ± | + | - | - | rcTnI 1-193 | 22 144 ± 8 # | 22 148 |
| | ~16 kDa | peak 2 | + | ± | ± | - | - | rcTnI 63-193 | 15 348 ± 15 ** | 15 337 |
| | ~15 kDa | peak 2 | + | - | - | - | - | rcTnI 73-193 | 14 130 **,†† | 14 096 |

* TnI products identified by their apparent molecular weights (Figure 9A).

† Immunological analysis (Western blots, Figures 9A, 11C) of protein products bound to Mabs: strong (+), weak (±), or no binding (-).

‡ Electrophoretic mobility in alkaline urea PAGE (Figure 11): mobile (+, TnC containing), non-mobile (-, not containing TnC).

§ The amino acid sequence(s) of proteins which are the theoretical best match to the observed masses.

// Best match to the observed masses was determined by calculating the mass of rcTnI, rcTnT, and mouse cTnC, sequentially clipped from the N- and C-termini using the PeptideMass tool from the Swiss Institute for Bioinformatics website.

¶ The source of the TnI products indicates the peak from RP-HPLC analyzed 8I-7 affinity column fractions of 60/45 tissue (Fig 4).

# Mass determined by electrospray mass spectrometry.

** Mass determined by matrix assisted laser desorption/ionization mass spectrometry.

†† The difference between the observed and theoretical masses is equal to that of a sodium ion (MW 35 Da), which is commonly found associated with mass spectrometrically analyzed proteins (as a result of the ionization process).